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Hypaxial Muscle Migration during Primary Myogenesis in *Xenopus laevis*

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In contrast to many vertebrates, the ventral body wall muscles and limb muscles of *Xenopus* develop at different times. The ventral body wall forms in the tadpole, while limb (appendicular) muscles form during metamorphosis to the adult frog. In organisms that have been examined thus far, a conserved mechanism has been shown to control migratory muscle precursor specification, migration, and differentiation. Here, we show that the process of ventral body wall formation in *Xenopus laevis* is similar to hypaxial muscle development in chickens and mice. Cells specified for the migratory lineage display an upregulation of *pax3* in the ventro-lateral region of the somite. These *pax3*-positive cells migrate ventrally, away from the somite, and undergo terminal differentiation with the expression of *myf-5*, followed by *myoD*. Several other genes are selectively expressed in the migrating muscle precursor population, including neural cell adhesion molecule (*NCAM*), *Xenopus* kit related kinase (*Xkrk1*), and *Xenopus* SRY box 5 (*sox5*). We have also found that muscle precursor migration is highly coordinated with the migration of neural crest-derived melanophores. However, by extirpating neural crest at an early stage and allowing embryos to develop, we determined that muscle precursor migration is not dependent on physical or genetic interaction with melanophores.

***Key Words: Xenopus*; ventral body wall; myoblast; melanophore; migration.**

INTRODUCTION

Skeletal muscles within the trunk region of vertebrates originate in the dermomyotome of the somites (Brand-Saberi and Christ, 1999). These muscles can be classified as either epaxial (forming the paraspinal and intercostal muscles) or the migratory hypaxial lineage (forming the limb and abdominal wall muscles). The development of the hypaxial muscle lineage has been described in chick, mice, and zebrafish (Christ *et al.*, 1983; Cinnamon *et al.*, 1999; Denetclaw and Ordahl, 2000; Kablar and Rudnicki, 2000; Neyt *et al.*, 2000). In all three organisms, the development of both limb/fin and body wall musculature occurs simultaneously, although by slightly different mechanisms. The hypaxial muscles originating from somites in the limb or fin fields undergo an epithelial-to-mesenchymal transition in the ventro-lateral lip of the dermomyotome before carrying out long-range migration into the limb/fin. In somites at interlimb/fin levels, however, hypaxial muscles undergo a short-range migration as extensions of the dermomyotome, maintaining their epithelial state.

In *Xenopus*, the development of limb and body wall muscles occur as two temporally distinct events. The ventral body wall forms during the primary development from egg to swimming tadpole, while the appendicular muscles form later during the metamorphosis of tadpole to adult frog (Jennings, 1992; Ko and Chung, 1997; Lynch, 1990). The separation of these events allows the study of ventral body wall formation in the absence of the added complexity of simultaneous limb muscle migration.

The molecular events associated with muscle migration have been well studied in both mouse and chick. The earliest specification of the migratory hypaxial musculature lineage can be seen as an upregulation of *pax3* in the ventro-lateral region of the somite (Bober *et al.*, 1994; Tremblay *et al.*, 1998; Williams and Ordahl, 1994). Pax3 alone has been shown to be a transcriptional activator of the muscle-specific transcription factor *myoD* (Maroto *et al.*, 1997). However, during migration, prospective muscle cells also express *msx-1*, a transcriptional repressor of *myoD* (Bendall *et al.*, 1999). When cells reach their final destination, they undergo terminal differentiation into skeletal

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muscle, beginning with the expression of *myf-5*, followed by *myoD* (Kablar *et al.*, 1998).

Mutations in the *pax3* gene have been identified in the *Splotch* mutant mouse (Epstein *et al.*, 1991). This mutation was originally identified in heterozygotes by aberrant pigmentation caused by neural crest defects. Homozygous *Splotch* mutants show considerable defects in neural crest and neural tube development, and, significantly, muscle precursors fail to migrate into the limbs. Ventral body wall muscles are present but are very disorganized and do not elongate to the full extent of wild-type embryos (Tajbakhsh *et al.*, 1997; Tremblay *et al.*, 1998). These results indicate a requirement for *pax3* expression in proper hypaxial muscle development.

Another major migratory population of cells is the neural crest (Christiansen *et al.*, 2000). In the trunk region of the *Xenopus* embryo, where ventral body wall formation occurs, the primary contributions of the neural crest are the dorsal root ganglia, motor neurons, and pigment cells, which migrate ventrally to cover the trunk of the embryo (Collazo *et al.*, 1993; Mayor *et al.*, 1999; Sadaghiani and Thiebaud, 1987). Pigment cells, or melanophores, migrate to the ventral region of the embryo by way of two distinct paths: the lateral path, which encompasses a route on the outside of the somites just underneath the epidermis; and the ventral path, between the somites and the neural tube (Epperlein *et al.*, 1996; Mayor *et al.*, 1999). The similar migratory defects in muscle and neural crest observed in the *Splotch* mutant suggest a possible conserved mechanism for migration.

In this study, we characterized the migration of myoblasts and the formation of the ventral body wall by examining the expression of several myogenic transcription factors. Although the expression of these genes has been presented in previous work (Frank and Harland, 1991; Hopwood *et al.*, 1991; Mariani *et al.*, 2001), their expression in migrating myoblasts has not been discussed or, in some cases, documented. The expression patterns of three other genes were examined due to observations that they may be expressed in migrating myoblasts (Balak *et al.*, 1987; C. Baker, personal communication). We have also examined the migration of melanophores and the relation of this process to that of ventral body wall formation. We found that ventral body wall formation in *Xenopus* occurs in a manner similar to that in other tetrapods. We have also found that the migration of melanophores is highly coordinated to that of myoblasts, both spatially and temporally, but is not required for their migration.

METHODS

General methods. *Xenopus laevis* embryos were generated and cultured by standard methods (Sive *et al.*, 2000). Embryos were allowed to develop in 0.3× Marc's modified Ringer (MMR) solution and staged according to the normal table of Nieuwkoop and Faber (1967).

Whole-mount in situ hybridization and antibody staining. Embryos were allowed to develop until the desired stage and then fixed for 2 h in MEMFA. *In situ* hybridizations were carried out with RNA probes labeled with either digoxigenin-UTP or fluorescein-UTP by using a multibasket technique previously described (Sive *et al.*, 2000). The muscle-specific 12/101 monoclonal antibody was used to visualize differentiated skeletal muscle (Kintner and Brockes, 1984). Undiluted monoclonal hybridoma cell supernatant was used following a standard immunohistochemistry procedure (Sive *et al.*, 2000). In cases where both *in situ* hybridization and 12/101 staining were carried out on embryos, *in situ* staining was performed first, followed immediately by immunohistochemistry.

Microsurgery. Embryos at stages 16, 21, and 24 were placed in agar-coated petri dishes containing 1× MMR. Forceps and insect pins were used to remove the dorsal-most tissues of the trunk region. Most of the neural tube was removed to prevent the regeneration of neural crest cells. Manipulated embryos were grown side-by-side with control unmanipulated embryos of the same clutch, so that they could be fixed at the appropriate stage. Fixed embryos were then either stained with the 12/101 antibody or *pax3* mRNA *in situ* probe.

Microinjection of DiI. A 0.5% solution of CellTracker CM-DiI (Molecular Probes), a chloromethylbenzamido derivative of DiI, was made up in 100% ethanol. Micropipets were pulled and broken to a tip of approximately 20 μm, backfilled with DiI solution, and attached to a Picospritzer (General Valve) so that DiI could be pressure injected. A Zeiss Axioplan microscope equipped with a rhodamine filter was used to observe the labeled cells. Embryos were immobilized while injecting and for viewing by immersion in 0.05% benzocaine solution.

RESULTS

The 12/101 Antibody Allows the Visualization of the Developing Ventral Body Wall

The 12/101 antibody, which is directed against differentiated skeletal muscle, was used to visualize the progression of muscle differentiation in the ventral body wall. At stage 31, differentiated skeletal muscle can only be seen in the somites (Fig. 1A). The first sign of hypaxial muscle in the ventral body wall occurs at stage 37, with the appearance of four clusters of differentiated tissue (Fig. 1B, arrows). By stage 41, the number of clusters has increased to a final number of eight, with each cluster continuing to increase in size in the ventral direction as development progresses (Fig. 1C). Previously, histological analysis had indicated seven muscle clusters (Lynch, 1990), but 12/101 staining has revealed a small additional cluster posterior to the originally described clusters. Stage 46 embryos show the final extent of the ventral expansion of the body wall muscles (Figs. 1D and 1E, arrows). Differentiated tissues do not meet at the ventral midline (Fig. 1E). A nonmuscular connection, the horizontal skeletogenous septum, is retained between the ventral body wall and the somite it arrose from (Fig. 1F, arrow head). Each original cluster of differentiated skeletal muscle retains segmental identity, as indicated by the lack of fusion of the myotubes between adjacent clusters (Fig. 1C, arrow heads).

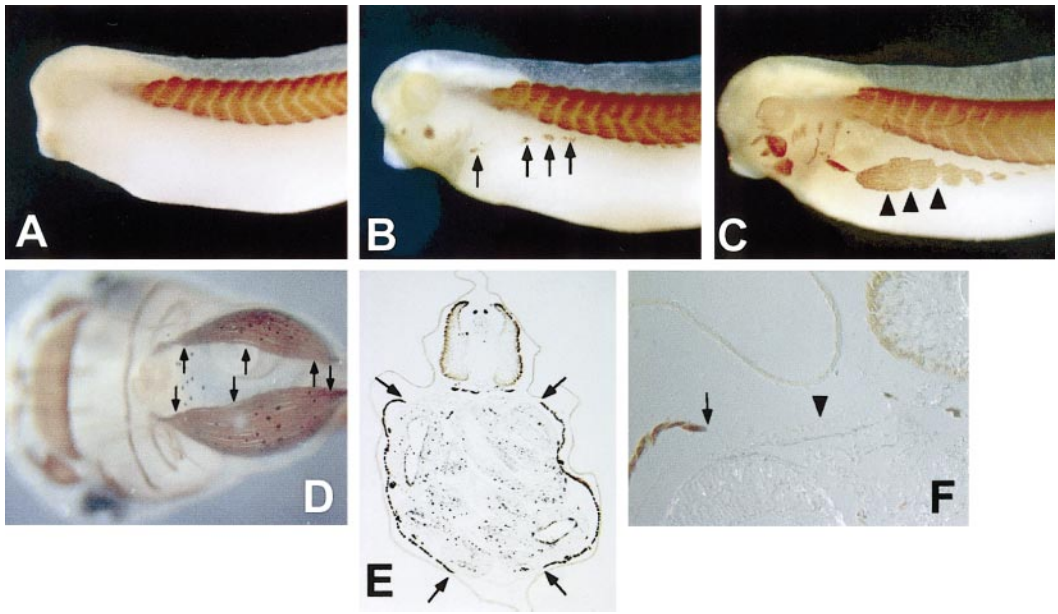


FIG. 1. Stage series showing the development of the ventral body wall. Immunohistochemistry was performed by using the 12/101 antibody on four different stages of embryos beginning with stages 31 (A), 37/38 (B), 41 (C), and 46 (D). The first appearance of differentiated ventral body wall muscle is at stage 37/38 (B), indicated by arrows. By stage 41, the ventral body wall has expanded ventrally and retains segmental identity, as indicated by the lack of fusion between adjacent myotubules (C, arrow heads). At stage 46, the ventral expansion of the body wall has ceased (D, arrows). The two sides of the ventral body wall fail to meet at the ventral midline (D). (E) A transverse section through a stage-46 embryo in the trunk region. Arrows indicate ventral and dorsal extent of the ventral body wall musculature. (F) A high-magnification view of a serial section to that shown in (E). The arrow marks the ventral limit of differentiated body wall, while the arrow head points to nonskeletal muscle tissue connecting the body wall to the somite it arose from. Embryos in (A–C) are oriented with anterior to the left and dorsal to the top of the page. Embryo in (D) is seen from a ventral viewpoint with anterior to the left, while in (E) and (F), dorsal is to the top of the page. In all subsequent figures embryos are seen with anterior to the left and dorsal to the top of the page except where otherwise noted.

Pax3 Expression Is Upregulated in the Ventro-Lateral Somite and Expands Ventrally

Evidence from chickens and mice has suggested a role for *pax3* in specifiying the hypaxial domain of somites. To determine whether a similar mechanism occurs during the formation of the ventral body wall in *Xenopus*, we stained embryos for *pax3* transcripts by whole-mount *in situ* hybridization. At stage 26, there is already a strong upregulation of *pax3* in the ventro-lateral region of the anterior somites (Fig. 2A, arrows). By stage 28/29, cell migration out of the anterior-most somites can be seen (Fig. 2B, arrows). As development proceeds, cells begin to migrate out of more posterior somites while those that have already begun to migrate continue moving ventrally. At stage 37/38, eight groups of cells can be visualized emerging from somites, corresponding to the eight clusters of differentiated muscle in the ventral body wall (Fig. 2D). Expression of *pax3* becomes weak by stage 41, just prior to the end of migration (Fig. 2E, arrows). In stage-37/38 and -41 embryos, *pax3* expression is found just ahead of 12/101 stained differentiated skeletal muscle, indicating that *pax3*-positive cells are in the undifferentiated state (Figs. 3A–3D).

Myogenic Transcription Factor Expression Correlates with Pax3-Expressing Cells

We next examined the expression of myogenic bHLH transcription factors *myf-5* and *myoD*. These mRNAs are expressed in paraxial mesoderm in an anterior-to-posterior progression, with *myf-5* preceding *myoD* by approximately 1 h (Hopwood *et al.*, 1991). While *myoD* expression is maintained, *myf-5* is extinguished, except for a minority of cells which we assume to be a pool of myoblasts (Hopwood *et al.*, 1991). At stages 26 and 28/29, *myf-5* expression is high in the ventro-lateral and dorso-medial lips of the somites, marking the expanding epaxial myotome (Figs. 2F and 2G). By stage 31, *myf-5*-positive cells can be seen at the ventro-lateral lips of the anterior somites, in a region corresponding to *pax3*-positive cells (Fig. 2H, arrows). This pattern continues throughout ventral body wall expansion. Expression of *myoD* initiates even later in the migrating muscle precursor cells; stained cells are not seen outside the somitic domain until stage 37/38 (Fig. 2N, arrows), and can never be seen directly emerging from the somites. At stage 41, *myoD* is expressed in broad stripes in the ventral body wall, with strongest expression in the ventral-most

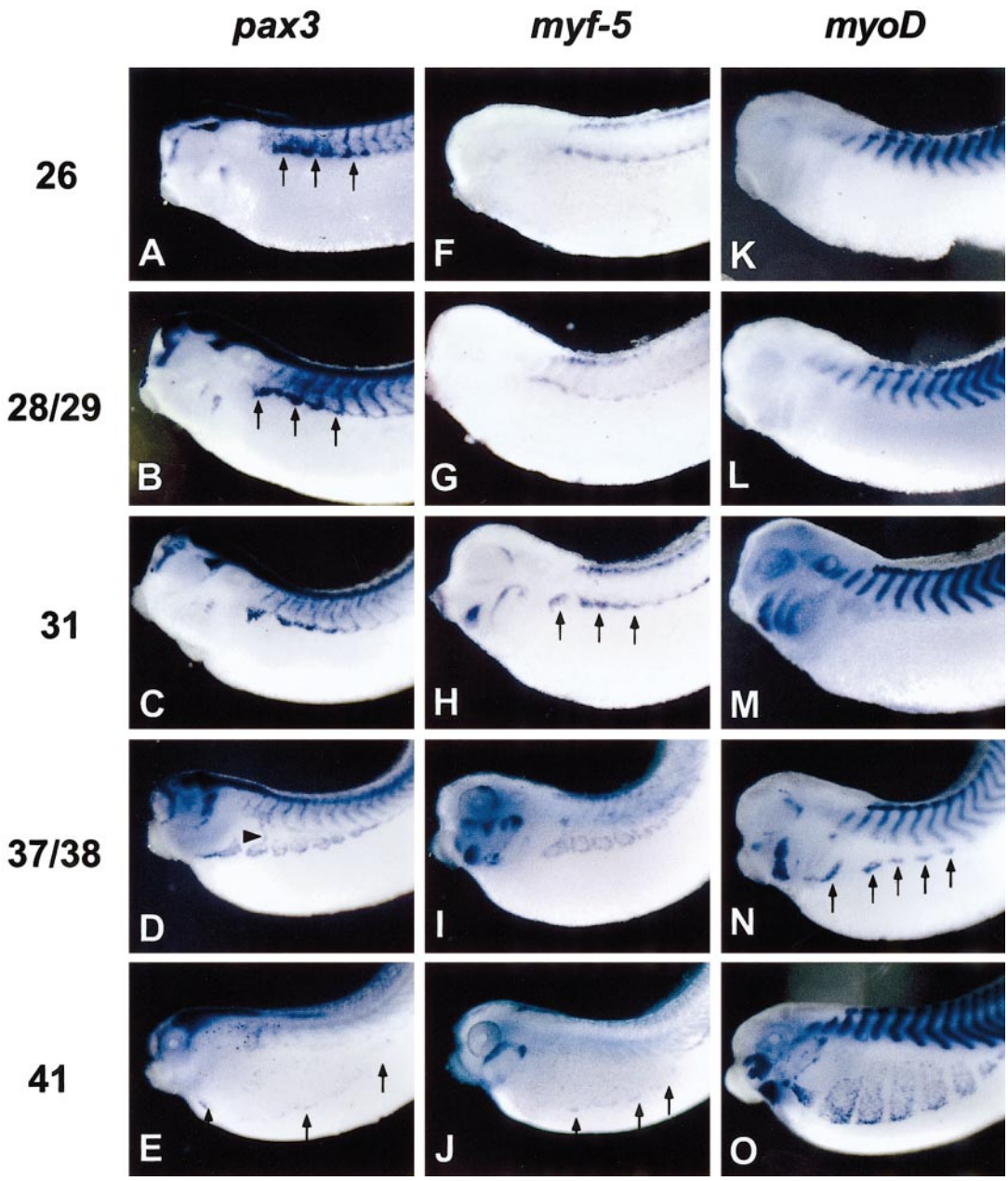


FIG. 2. Expression patterns of the myogenic transcription factors *pax3*, *myf-5*, and *myoD*. Upregulation of *pax3* in the dorso-lateral lip of the anterior trunk somites is apparent at stage 26 (A, arrows). By stage 28/29 *pax3*-positive cells can be seen leaving the somites and migrating ventrally (B, arrows). At stage 37/38, *pax3* cells can be seen at the front of the migration (D) as they continue to stream out of the somites (D, arrow head). Stage-41 embryos have faint *pax3* expression at the front of the migrating cells (E, arrows). Early *myf-5* expression is restricted to the dorso-medial and ventro-lateral lips of the somites, marking the expanding epaxial myotome (F, G). At stage 31 (H, arrows), *myf-5*-expressing cells can clearly be seen outside of the somitic region migrating ventrally, and continuing to do so through stages 37/38 (I) and 41 (J, arrows). Expression of *myoD* is present only in the somitic region (K–M) until stage 37/38 (N), when it appears in discrete patches clearly separate from the somites (arrows). By stage 41, there are long stripes of *myoD* expression corresponding to the differentiated ventral body wall muscle (O). Expression is greatest in the ventral-most region of the body wall, where skeletal muscle has most recently differentiated.

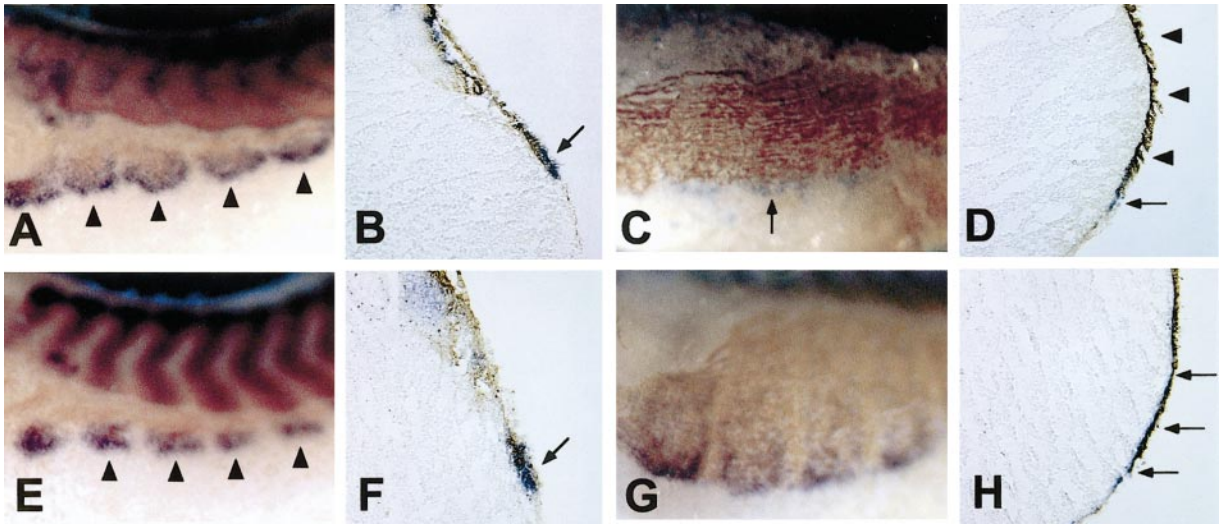


FIG. 3. Embryos stained for either *pax3* (A–D) or *myoD* (E–H) followed by 12/101 staining. Stage-37 embryos stained for *pax3* expression (A, B) reveal the *pax3*-expressing cells are just ventral to differentiated muscle (arrow heads), with little overlap, as indicated from a transverse section (B) of embryo in (A) (arrow). The lack of overlap is more dramatic in stage-41 embryos (C, arrow). (D) A transverse section of embryo in (C). The arrow shows *pax3* expression which does not overlap with 12/101 staining (arrow heads). Stage-37 embryos stained for *myoD* (E, F) indicate significant overlap of *myoD* expression and differentiated muscle (arrow heads in E, arrow in F). At stage 41, overlap of *myoD* expression continues over a broad range of differentiated muscle (G), which can also be seen in (H) (arrows). In (B), (D), (F), and (H), dorsal is toward the top of the page.

region (Fig. 2O). The expression of *myoD* overlaps with 12/101 staining, indicating that those cells expressing *myoD* have terminally differentiated into skeletal muscle (Figs. 3E–3H).

Other Genes Expressed in the Migrating Muscle Precursor Population

Results of previous work suggested that other genes would be expressed in the migrating myoblasts (Baker *et al.*, 1995; Balak *et al.*, 1987), so we reexamined their expression patterns at appropriate stages. Of the three genes examined during the period of ventral body wall formation, all showed strong expression in the migrating cell population. Neural cell adhesion molecule (*NCAM*), which has previously been shown to be expressed in the neural tissue, is expressed in the migrating cells with a ventral extent similar to *pax3*. Staining appears more diffuse across the migratory plane, especially at stage 37/38 (Figs. 4A–4E).

Xenopus kit related kinase (*Xkrk1*), a gene closely related to the vertebrate *c-kit* gene, has previously been described as being expressed in the developing lateral line system. Here, we find that, in addition to the reported staining, there is also strong staining in the migrating myoblasts. The expression of *Xkrk1* is unique in comparison to the other genes expressed in the migrating cells in that it is only seen in those cells that have left the somite, and is not present in the premigratory cells of the ventro-lateral lip of the somite (Figs. 4G and 4H, arrows). Staining in the migrating cells is strongest at stage 37/38 and is undetectable by stage 41. With the exception of the ventro-lateral lip of the somites, expression of *Xkrk1* seems to overlap with *pax3* staining in the migrating cells (Figs. 4F–4J).

A third gene, *sox5* (T. Grammer, unpublished results), a member of the Sox family of transcription factors, has been shown here to be expressed in the migrating cells in a manner reminiscent of *myoD* staining. *sox5* is expressed heavily in both neural and somitic tissues. Its expression in migrating hypaxial musculature is similar to *myoD* staining (Figs. 4K–4O, arrows), and overlaps with 12/101 staining of differentiated muscle (data not shown). In addition, recent reports have also shown that *six1*, *six2*, and *eya1* are expressed in migrating ventral body wall muscles (David *et al.*, 2001; Ghanbari *et al.*, 2001).

Trunk Somites I-VIII Contribute to the Ventral Body Wall

The eight clusters of cells that migrate to form the ventral body wall originate from trunk somites I-VIII. During *Xenopus* somitogenesis, four head somites form, followed posteriorly by the formation of the trunk somites. The head somites gradually disintegrate as the tadpoles develop. By stage 31, the first head somite has completely disintegrated, while head somites 2 and 3 are significantly reduced in size (Nieuwkoop and Faber, 1967). A combination of 12/101 antibody staining and *pax3 in situ* hybridization on stage-31 embryos reveals that the head somites

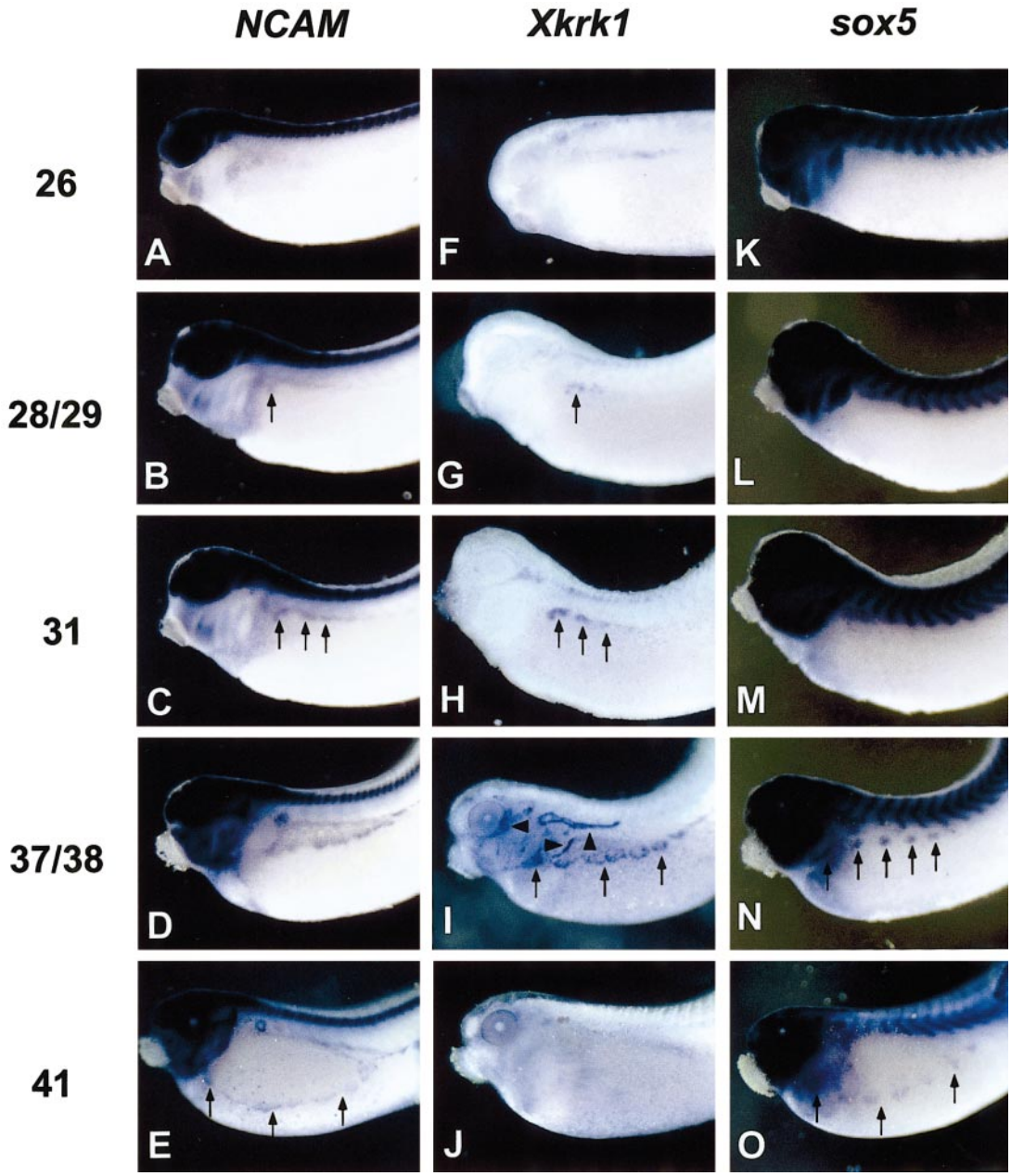
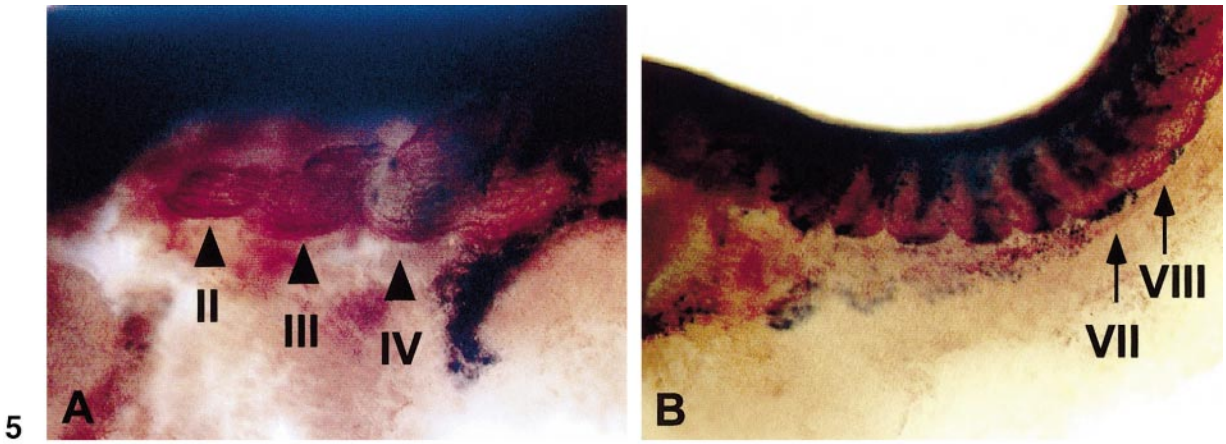


FIG. 4. Expression patterns of *NCAM*, *Xkrk1*, and *sox5*. Heavy *NCAM* expression can be seen in the neural tissues of the head and neural tube throughout the stage series (A–E). *NCAM*-expressing cells can be seen moving out of the somites at stage 28/29 (B, arrow) and continuing to move ventrally at later stages (C–E, arrows). At stage 37/38, expression can be seen from the leading edge of the migrating cells all the way back to the somites (D). At stage 41, *NCAM*-expressing cells are present at the leading edge of migration (E, arrows). The expression of *Xkrk1* can be seen in the developing lateral line system, particularly at stage 37/38 (I, arrow heads). Expression is also found in the migrating myoblasts that have left the somite, beginning at stage 28/29 (G, arrow), and continuing to move ventrally through stage 37/38 (I, arrows), where expression in the developing ventral body wall is particularly strong. By stage 41, there is no longer any expression of *Xkrk1* in the ventral region of the embryo (J). The expression of *sox5* can be seen in neural tissue and somitic mesoderm throughout the stage series (K–O). Its expression in the ventral body wall is similar to *myoD* expression, first appearing in small clusters of cells at stage 37/38 (N, arrows) and moving ventrally at stage 41 (O, arrows).



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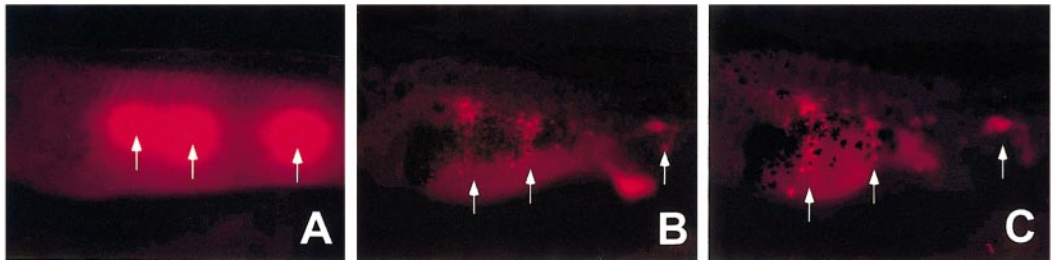

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FIG. 5. Trunk somites I-VIII contribute to the ventral body wall. Embryos were stained for *pax3* followed by 12/101 antibody staining. A stage-31 embryo (A) shows that the three remaining head somites (arrow heads) do not contribute *pax3*-positive cells to the ventral body wall. A stage-36 embryo (B) shows that trunk somites VII and VIII (arrows) are the last somites to contribute to the ventral body wall.
FIG. 6. DiI labeling of migrating myoblasts (A–C). Arrows mark three points of injection along the anterior–posterior axis targeting the ventro-lateral region of the somites (A). As development of this embryo proceeds, cells labeled by the anterior-most injection migrate first and farthest (B, C, arrows). The cells labeled by the posterior-most injection in the tail region show very little migration.

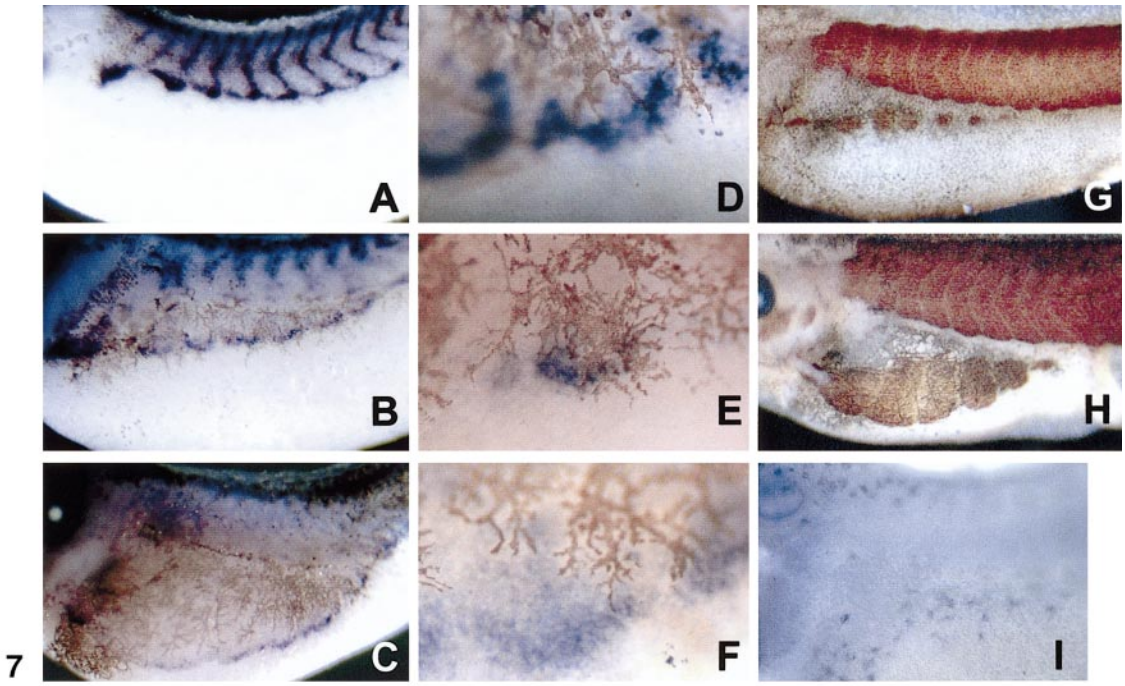
do not contribute to the *pax3*-positive cells that migrate to form the ventral body wall (Fig. 5A, arrow heads). The first somite that exhibits *pax3*-positive cells migrating ventrally is trunk somite I. Analysis of stage-37/38 embryos indicates that trunk somites I-VIII contribute *pax3*-positive cells to the ventral body wall (Fig. 5B, arrows).

Visualization of Migrating Cells

DiI labeling was used to ensure that the patterns of gene expression reflect migration, and are not simply a wave of gene expression. Embryos were injected with DiI three times each on one side along the anterior–posterior axis (Fig. 6A, arrows). Injections were targeted to the ventro-lateral region of the somites. Figures 6A–6C show various developmental stages of an injected embryo. In accordance with myoblast migration, the anterior-most labeled cells migrated first and farthest toward the ventral region of the embryo. The posterior-most labeled cells in the tail region of the embryo showed very little movement, consistent with the migration of cells from the somite (Figs. 6B and 6C, arrows). To determine the extent to which neural crest cells contribute to the labeled, migrating cells, we injected DiI into the dorsal neural tube in the trunk region of embryos. Very little contribution of these cells to the ventral trunk region can be seen (data not shown).

Hypaxial Muscle Precursors Comigrate with Neural Crest-Derived Melanophores

One of the major lineages of neural crest cells in the trunk region of the embryo is that of the melanophores or pigment cells. These can be visualized migrating ventrally from the dorsal side of the embryo. Melanophores can first be seen posterior to the somites in the anterior trunk at approximately stage 33/34, in a region where *pax3*-expressing cells are migrating out of the somite (data not shown). At stage 37/38, the front of melanophore cells is highly coordinated with *pax3*-expressing cells throughout the trunk region (Fig. 7B). By stage 41, they are still highly coordinated in the mid to posterior trunk region, but in the anterior trunk the melanophores have passed the *pax3*-expressing cells and migrated further ventrally (Fig. 7C). High magnification views of *pax3*, *myoD*, and *NCAM* staining in stage-37 embryos shows the highly coordinated position of gene expressions with pigment cells, and also



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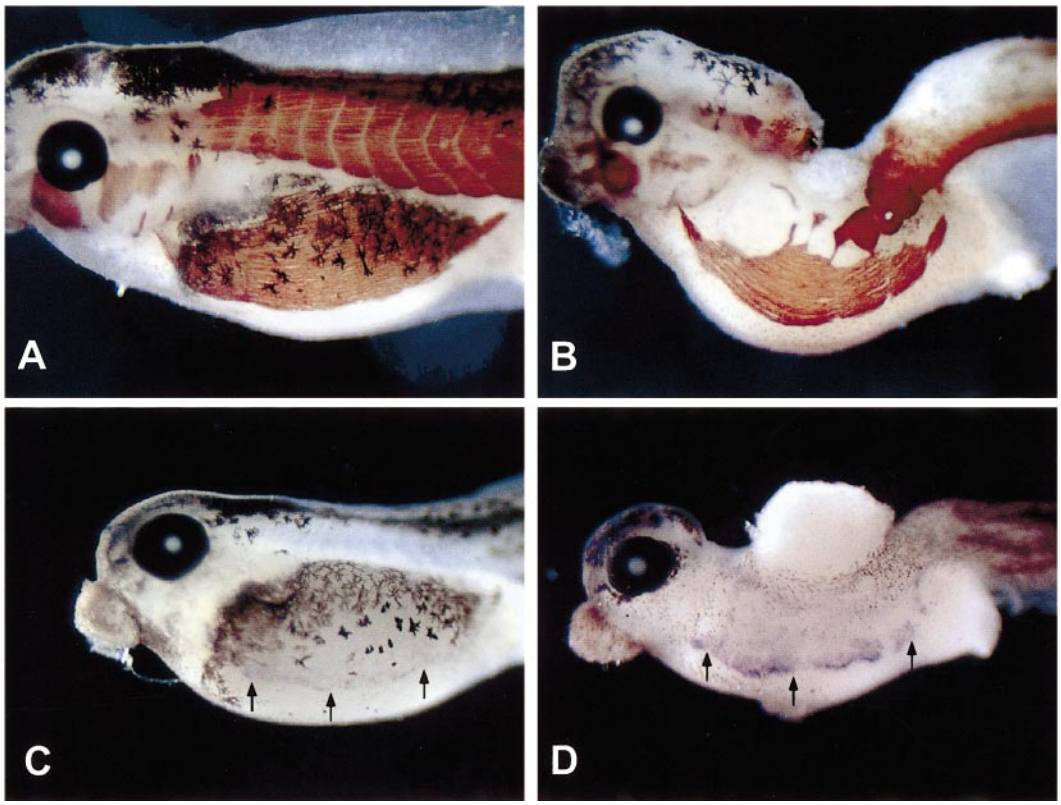

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FIG. 7. Neural crest-derived melanophores comigrate with muscle precursors. Expression of *pax3* at stages 31 (A), 37/38 (B), and 41 (C) in unbleached embryos. (B, C) Comigration of *pax3* staining cells with pigmented melanophores can be seen. Close-ups of *pax3* (D), *myoD* (E), and *NCAM* (F) expression in stage-39 embryos showing close spatial and temporal movements of melanophores and myoblast precursors. Melanophores can be seen overlying the stained cells. 12/101 staining in stages 37/38 (G) and 41 (H) also matches the ventral extent of the melanophores. Expression of *slug* in a stage-37 embryo marks melanophores in the ventral trunk region and in the head (I).

shows that pigment cells overlie the stained cells (Figs. 7D–7F). 12/101 staining also demonstrates the highly similar spatial and temporal expansion of the ventral body wall musculature and the melanophores (Figs. 7G and 7H). Differentiated melanophores can also be identified by *slug* expression, a previously unreported domain for this transcript (Fig. 7I). In unbleached embryos, *slug* expression cannot be seen in the trunk region. However, once bleached, *slug* expression can be seen in cells that were previously pigmented, with a stellate staining pattern similar to melanophore morphology.

Neural Crest Cells Are Not Required For Hypaxial Muscle Migration

Due to the highly coordinated migration of the hypaxial musculature and melanophores, we considered the possibility that the muscle precursor cells require signaling or direct interaction with the migrating neural crest in order to leave the somite and/or migrate ventrally. To address this concern, we removed the entire dorsal region of the trunk at stages 16, 21, and 24 (stages 21 and 24 not shown), in order to eliminate trunk neural crest and prevent its regeneration. Manipulated embryos were allowed to develop until stage 40 or beyond and examined for the presence of differentiated ventral body wall muscle and migratory muscle precursors by means of 12/101 staining (Figs. 8A and 8B) and *pax3 in situ* hybridization (Figs. 8C and 8D). In every case where melanophores were absent from the trunk, muscle precursors migrated and differentiated normally (Fig. 8). Thus, muscle migration from the somites into the ventral body wall does not require simultaneous crest migration.

We also examined the expression of *NCAM*, *Xkrk-1*, and *sox5* in manipulated embryos. While the expression patterns of these three genes suggest that they mark migrating muscle cells, they may instead be marking closely associated migrating neural crest cells. All three genes were expressed in the ventral body wall region similar to *pax3* expression in manipulated embryos, indicating that these genes mark migrating muscle cells (data not shown).

DISCUSSION

Ventral Body Wall Formation in Xenopus

The two-step indirect development of *Xenopus* allows ventral body wall formation to be observed without the added complexity of simultaneous appendicular muscle migration and formation. 12/101 staining indicated that the body wall muscle forms well before the onset of metamorphosis and limb bud initiation. The analysis of myogenic transcription factor expression within the developing body wall is consistent with studies in the chick and mouse, and recapitulates evidence that *pax3* expression precedes that of *myf-5* and *myoD*, and *myf-5* is expressed earlier than *myoD* during muscle differentiation (Brand-Saberi and Christ, 1999; Dietrich, 1999; Tajbakhsh *et al.*, 1997). The temporal cascade of gene expression of differentiating myoblasts can be seen spatially during the formation of the body wall. Those cells at the front of migration are at the "early," undifferentiated state, and express *pax3*. At approximately the same position as the *pax3*-positive cells are *myf-5*-expressing cells, which represent the earliest marker for terminal differentiation into skeletal myoblasts (Figs. 2F–2J) (Hopwood *et al.*, 1991). Slightly dorsal to the *pax3*- and *myf-5*-positive cells are the *myoD*-expressing cells, a later marker of terminally differentiated myoblasts (Figs. 2K–2O) (Rawls and Olson, 1997).

Despite the expression patterns of *pax3* and *myoD*, the regional specification of hypaxial muscles within *Xenopus* somites remains unclear. *Xenopus* dermatome has been identified as a thin layer of cells on the lateral surface of the somitic mesoderm, but few data have been presented on the specification and behavior of this tissue (Keller, 2000). Analysis of both whole-mount and sectioned embryos has revealed that *pax3* is expressed in both anterior and posterior domains of each somite, while *myoD* is expressed in the center of each somite. In sectioned embryos (data not shown), *pax3* expression is found in the lateral portions of each somite, but these cells cannot be unambiguously assigned to dermatome. Our data therefore cannot conclusively determine the origin of hypaxial muscles in *Xenopus* somites.

Role of NCAM, Sox5, and Xkrk1 in Migrating Muscle Precursors

The expression of genes other than the myogenic transcription factors raises questions as to what their role may be in this migrating cell population. The expression of *NCAM* in muscle precursor cells has been proposed to be involved in the aggregation of these cells and the fusion of myoblasts to form myotubules (Charlton *et al.*, 2000; Dickson *et al.*, 1990; Knudsen *et al.*, 1990). It may also function in axonal bundling, migration of neurons, muscle innervation, and synaptogenesis. At early tadpole stages, *NCAM* expression is not present in the myotome of the somite.

FIG. 8. Removal of neural crest does not inhibit migration and differentiation of ventral body wall musculature. 12/101 (A, B) and *pax3* (C, D, arrows) staining in control (A, C) and manipulated (B, D) embryos. Complete lack of melanophores in the ventral region of the embryos (B, D) indicates that this neural crest lineage has been successfully removed. Differentiated body wall musculature (B) and migrating *pax3*-expressing cells (D, arrows) are present in manipulated embryos despite the lack of melanophores.

However, migrating myoblasts express *NCAM* at these stages, identifying a possible role for *NCAM* in the migration or prospective fusion of these cells. The widespread distribution of *NCAM* expression over the migratory range of muscle precursors suggests a prominent role in the formation of the ventral body wall and/or the formation of the peripheral nervous system (Figs. 4D and 4E).

The role of *sox5* in development is not well understood. In *Xenopus*, *sox5* is expressed early in paraxial mesoderm and neural crest (T. Grammer, personal communication). Here, we show it is also expressed in the migrating myoblasts. The similarity in expression between *sox5* and *myoD* in the somites and ventral body wall suggests that *sox5* is involved in the late differentiation of skeletal muscle (Figs. 2K–2O and 4K–4O).

Previously, the expression of *Xkrk1* had only been reported in the developing lateral line system (Baker *et al.*, 1995). Here, we have found that *Xkrk1* is expressed in cells migrating to form the ventral body wall in a pattern very similar to *pax3*. Its expression is unique in that it is only found in those cells that have left the somite and begun migrating ventrally to form the ventral body wall (Figs. 4F–4I). By stage 41, when *pax3* expression is still detectable in migrating muscle precursors, there is no detectable expression in the ventral body wall region (Fig. 4J). A relative of *Xkrk1*, *c-kit*, has been shown to be required for melanophore survival and migration in zebrafish (Parichy *et al.*, 1999, 2000). *c-kit* binds the ligand stem cell factor, which has also been shown to cause cell proliferation and migration (Mackenzie *et al.*, 1997). *Xkrk1*, which is related to *c-kit* but considered not to be the true orthologue, may be playing a similar role to *c-kit*, but in the muscle precursors of the ventral body wall and not the melanophores.

Neural Crest Cells Are Not Needed for Muscle Precursor Migration and Differentiation

The highly coordinated migration of muscle precursors and the neural crest-derived melanophores suggested that there may be an important interaction between the two cell types in order for migration to proceed. The removal of the neural crest at an early stage allowed us to determine whether or not this was the case. The normal migration and differentiation of muscle cells despite the complete lack of melanophores demonstrates that an interaction between the neural crest and muscle precursors is not required for initiation and subsequent migration and differentiation of the ventral body wall musculature in *Xenopus* (Fig. 8). This result, combined with the simultaneous migration of two cell populations in normal embryos, suggests that a common signal may act upon both cell populations to initiate and drive cell migration along a dorso-ventral path.

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